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STUDIES ON MICROSPORIDIA PARASITIC IN MOSQUITOES

II. ON THE EFFECT OF THE PARASITES UPON THE HOST BODY *

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In a former paper (Kudo, 1921), I have described two new Microsporidia parasitic in mosquitoes collected at Urbana, Illinois. Due to the small number of infected insects found, I could not carry through the investigation.

During the summer of 1920, I studied several cases of microsporidian infections in mosquitoes in Pennsylvania and New York. Observation both in the field and in the laboratory brought to light imperfectly known points in the previous paper. In later papers I shall attempt to present the results of observations thus made. I am greatly indebted to Doctor H. G. Dyar of Washington, D. C., who knidly identified the species of mosquitoes dealt with herein.

OCCURRENCE OF MICROSPORIDIA IN MOSQUITOES

In the review of the literature dealing with the microsporidian parasites of mosquitoes of Europe, I (1921) failed to mention the cases recorded by Grassi and Ross.

Grassi (1901) speaks of two kinds of parasites in mosquitoes of the genus Anopheles. One of the forms was noticed in the general body cavity of the host. It was either free or attached to organs such as the intestine, salivary gland and dorsal vessel. In a probable young stage of the free form, it was a rounded multinucleate protoplasmic mass. On two occasions it contained oval bodies, each with a brilliant central corpusle and capable of escaping from the mass. But when the parasite is attached to the host organs which condition is more commonly found than the free state, it appears tubular or irregularly globular. It becomes surrounded by a membrane and the contents break up into a large number of spores each provided with a membrane. The presence of a polar capsule in the spore could not be determined. The second parasite invades especially eggs. In the protoplasmic mass one finds two, four and eight nuclei. A capsule is formed when eight sporozoites are produced in the mass. From this description and the figures given by Grassi, one cannot decide whether the said author saw Microsporidia or not. I have in vain tried to secure a statement and preparations from this investigator.

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Ross (1906) states that he saw "spores of a protozoal parasite (in India in 1898), consisting of a collection of exactly eight spores closely packed within an oval envelope, which was probably the shell of the cell which contained the parent cytozoon. Each spore was oval, measuring 2 by 5μ, refractive, apparently hard, and containing a circular vacuole at one focus of the ellipse. Numbers of these little clusters of spores were found within the sheath of the ventral nerve trunk of the imago, especially in a small species of Stegomyia occurring near Ootacamund, and also, as far as I remember, in *C. fatigans* of Ootacamund itself. The mature spores appeared to escape in an unknown manner from the imago, and could be found in the water at the bottom of the vessel in which the insects were kept. There they seemed to grow much in size; and on several occasions I thought I observed amoebic embryos escaping from them. Since that time I have never seen these bodies again. They were not the same, I think, as the Myxosporidia with eight sporozites mentioned by Grassi, and also, apparently by Christophers and Stephens." Although no figures accompany the above quoted statement and the presence of a polar filament in the so-called spore is not spoken of, I am inclined to think that Ross probably saw a Microsporidian. It is of special interest that the nerve cord of an adult mosquito becomes infected by the parasites.

Quite recently three German authors reported briefly the occurrence of microsporidian parasites in the mosquitoes which they studied. Bresslau (1919) states that in a smear of the contents of body cavity of a larva of *Culex pipiens,* he found a microsporidian, *Nosema culicis,* the spores of which measure 4.5 to 5.5μ long by 1.8 to 2.4μ broad, and that he once saw another Microsporidian apparently closely related to the genus Thelohania in a larva of *Culiseta* (*Theobaldia*) *annulata.* Nöller (1920) figures stages in the sporogony of *Thelohania* sp., which were present in large numbers in the fat bodies of a larva of *Aëdes nemorosus* obtained in the vicinity of Hamburg in the spring of 1919. He did not find mature spores and expressed his view that it was probably identical with *Thelohania legeri* Hesse. The same author further states that *Nosema* sp. was found in the larvae of *Aëdes nomorosus* as well as *A. cantans* and that its somewhat broader shape suggested that it was not identical with *Nosema culicis.* Martini's valuable paper on mosquitoes (1920) does not contain any original observations except that *Nosema* sp. was found in *Aëdes* sp. in the vicinity of Hamburg, which is similar to the above quoted observation of Nöller.

The intensity of microsporidian infection in mosquitoes has not been given by European invstigators except Hesse (1904); it therefore would appear from the statement quoted in this and the former reports that microsporidian parasites are either rare or very fragmentarily studied in European mosquitoes. In contrast with this, my observations during 1919 and 1920 show that the Microsporidian parasites are commonly found in mosquitoes in some parts of the United States.

The examination of mosquitoes of Pennsylvania and New York last summer revealed two facts among many other interesting matters: first, the microsporidian infection of mosquitoes takes a serious course, and second, Microsporidia occur rather widely among different species of mosquito on this continent.

The two Miscrosporidia, *Thelohania magna* and *T. illinoisensis*, which I described from Illinois, have been found to occur also in other localities. The former was found in *Culex territans* in Pennsylvania and the latter in *Anopheles qudrimaculatus* in New York. In the latter locality a new hitherto unrecorded Microsporidian was seen in larvae of *Culex apicalis*, to which the name *Thelohania opacita* nov. spec. is given.

GEOGRAPHICAL DISTRIBUTION OF MICROSPORIDIA IN MOSQUITOES

The following list includes all the microsporidian or Microsporidia-like parasites of mosquitoes of the world known up to the present:

Europe:	
Thelohania legeri	Larvae of *Anopheles maculipennis;* France (Hesse, 1904).
Thelohania sp.	Larva of *Culiseta annulatus;* Germany (Bresslau, 1919).
Thelohania sp.	Larva of *Aëdes nemorosus;* Germany (Nöller, 1920).
Nosema culicis	Larva of *Culex pipiens;* Germany (Bresslau, 1919).
Nosema sp.	Larva of *Aëdes nemorosus* and *A. cantans;* Germany (Nöller, 1920).
Nosema sp.	Larva of *Aëdes sp.;* Germany (Martini, 1920)
Ambiguous forms:	
Glugea (?) sp.	Larvae of *Culex* sp.; Germany (Pfeiffer, 1895).
Nosema sp.	Host species not mentioned. Christophers and Stephens (1908).
Glugea (?) sp.	Adult of *Anopheles* sp.; Italy (Grassi, 1900).
Thelohania (?) sp.	Adults of *Anopheles* sp.; Italy (Grassi, 1900).
Asia:	
Thelohania (?) sp.	Adults of *Culex fatigans* and *Stegomyia* sp.; India (Ross, 1906).
South America:	
Glugea (?) *stegomyiae*	Larvae and adults of *Aëdes calopus:* Marchoux, Salimbeni and Simond (1903).
North America:	
Thelohania magna	Larvae of *Culex pipiens* and *C. territans;* United States (Kudo, 1920, 1921).
T. illinoisensis	Larvae of *Anopheles punctipennis* and *A. quadrimaculatus;* United States (Kudo, 1921).
T. opacita	Larvae of *Culex apicalis;* United States (Kudo, 1921).

Of these writers, Hesse made an effort to study the effect of the parasite upon the host larvae, and suggested that the infected larva did not suffer from the infection.

The results of my own observations upon three North American Microsporidia are as follows:

Thelohania magna in *Culex territans*

A large number of *Culex territans* were found breeding in an old boat filled with rain water on Conewango Creek at Warren, Pennsylvania. Thirty-two larvae were collected on July 5, 1920. Microscopical examination showed that five of them were heavily infected by a species apparently identical with *Thelohania magna*. On July 10 more material was collected from the same place. Nine out of 193 living larvae and three out of 12 dead larvae were found to be heavily infected by the same Microsporidian. The infection was very much heavier than in the case of *Culex pipiens* and consequently the host animals showed a striking difference from the normal ones. In the case of *Culex pipiens,* I have stated (1921), that the larvae "did not show any marked difference in their activity" and that "some of the larvae appeared to be more opaque whitish, with more or less distended thorax, than the majority." The heavily infected larvae of *Culex territans* were uniformly much smaller in size than the normal ones. At first I thought that these smaller infected larvae hatched out from eggs which were laid later than those which gave rise to healthy ones. Yet I did not find normal larvae of smaller dimensions in the collected material. It seems probable that the decrease in size of the infected larvae is due to the presence of the Microsporidian. A similar condition is found in silk-worms, *Bombyx mori,* infected by *Nosema bombycis.* A single batch of eggs laid by a moth give rise to worms of variable size according to the degree of infection by the parasites. The difference in size between normal and infected worms is frequently so great that it is hard unless one traces the larval development to conceive that they hatched out from eggs laid by one and the same moth.

The opacity of the body of the infected larvae was striking. The heavy infection could easily be determined under a magnification of about 10 diameters by virtue of milky opaque and whitish color with a slightly yellowish tone. Although the infected larvae of *Culex pipiens* did not show any marked decrease in activity, several of the heavily infected larvae of *Culex territans* were far less active than the normal ones. When kept in an aquarium, they usually stayed on the bottom without any active movements. When they came up to the surface of the water, the head was almost always directed upwards and they stayed in this position at the surface until disturbed. When disturbed the normal ones swam down very swiftly, while the diseased ones would start this motion very slowly. In a few seconds the normal larvae would come up again to the surface, while the heavily infected ones would not do so for some length of time.

On July 10 the debris on the bottom of the boat was collected and twenty-two dead larvae were found completely filled with the Microsporidian spores. Among various Protophyta and free living ciliates and flagellates, fragments of infected larval tissues and apparently normal ones together with scattered mature spores were recognized.

Rearing Experiment No. 1.—Thirty-six larvae from the collection made on July 5 were kept in a glass jar, the bottom of which was covered with garden soil. The dead larvae were subjected to daily microscopical examination for the schizonts and spores of the Microsporidian. On July 7 three larvae were found motionless on the bottom. One was entirely dead and showed no microsporidian parasites. The other two perfectly motionless when handled by a pipette, exhibited a very weak muscular contraction under the microscope. Both were heavily infected by young and mature sporoblasts and free spores in the adipose tissue and body cavity. The anal gills were filled with scattered spores which could be seen under a low magnification. On July 8 one larva was found dead. It was heavily infected and the body was completely filled with the spores, exhibiting the typical opacity. Later, two, nine, two, two and five larvae were found dead on July 9, 10, 11, 12 and 13, respectively; these did not show any degree of microsporidian infection. From the larvae seven pupae developed on July 8 to 10. In two of them it was found that one spore was attached to the external surface of the head and thorax, but not in the interior of the body. The remaining pupae did not show any stages of parasites. The five live larvae examined on July 14 were free from the infection. From the above it is seen that three out of thirty-six larvae were heavily infected by the Microsporidian and that the infected larvae died in from two to three days in a rearing jar.

Rearing Experiment No. 2.—Seventy larvae out of 184 apparently normal collected on July 10 and taken at random were kept in a glass jar with clean water and bottom. Due to an unfortunate early departure from the place, the observations could not be extended beyond July 14, on which date all the living larvae were examined. On July 13, four were found dead, one of them was heavily infected. Not one of the living larvae examined on the next day contained any recognizable stage of the parasite. In this case it appears that one out of 70 larvae was infected and that 12 pupae from these larvae were free from the Microsporidian.

Eighty-five pupae collected from the water in the boat on July 10 were kept under observation in a glass jar. On July 11, 13 and 14, five, eight and five pupae were found dead. They were not infected by the Microsporidian nor were the sixty-seven adults that emerged from the pupae. It thus appears that none of the pupae or adults examined were attacked by the parasites.

Thelohania illinoisensis in *Anopheles quadrimaculatus*

Six and forty-eight larvae of *Anopheles quadrimaculatus* were collected from a creek at Spring Valley, New York, on August 18 and 21, 1920, respectively. Two individuals from the first and one from the second collection were found to be infected by a species of Microsporidia apparently identical with *Thelohania illinoisensis.* Two of the infected larvae showed a typical opaque white coloration of the body. One of them was so strikingly opaque in color and inactive that its diseased condition could be diagnosed as due to some microsporidian parasites while it was still in the water. It died in about thirty minutes after capture. In the second infected individual it was distinctly noticed that the adipose tissue had been infected and that the rupture of the membrane of the infected host cells had scattered various developmental stages of the parasite through the general body cavity. The third infected larva did not show any opacity in the body; it however contained numerous young octosporous sporoblasts in the adipose tissue. Three pupae collected from the same place were free from the infection.

No rearing experiments were conducted due to the small number of the larvae that could be collected, but it seemed probable that the Microsporidian multiplies rapidly and as a result kills the host larvae before they transform into pupae.

Thelohania opacita in *Culex apicalis*

Among fifty-one larvae of *Culex apicalis* collected in a small creek at Spring Valley, New York, and examined on from August 11 to 28, three were found to be infected by a new Miscrosporidian for which the name *Thelohania opacita* is proposed. The opaque appearance of the body of some suggested at the time of collection probable protozoan infection. The larvae were kept in different jars as they were collected and the dead ones were examined microscopically as in other cases. The infected ones died on the second, third and fourth days while normal ones died from the third to the tenth days after capture, some having become pupae during this period. Forty-five adults were collected from the surface of the water and from jars; no stages of the Microsporidian could be found in them. Thus it seems probable that the effect of the infection upon the host is lethal and the infected larvae usually succumb before pupation.

The most conspicuous symptom of the infection is a striking opacity of the body. Two of the infected larvae were opaque white throughout; the third was partially infected as shown here (Fig. A). Decrease in activity was also noticeable as in other cases, although no recognizable difference in size could be detected between healthy and infected larvae.

The octosporous sporoblast is mostly rounded, measuring on an average 11.5 by 8.5μ. Its membrane is very delicate, and can be seen only in places as a faint line in fresh condition. It easily breaks up under a slight pressure. Frequently tetrasporous sporoblasts containing spores much larger than those found in octosporous sporoblasts were seen. The two kinds of spores differ only in dimensions, their structure apparently being the same. It seems probable that these spores correspond to microspores and macrospores of other species reported by several European authors (Kudo, 1916). I do not find any evidence which might justify the view presented by Vaney and Conte (1901) concerning the significance of these two kinds of spores. On the other hand, the statement I made in a former paper (Kudo, 1916) appears to be supported by the present species. In my opinion the nucleus of the sporoblast ordinarily divides three times producing eight nuclei, while under certain conditions, the nucleus divides only twice producing four nuclei. The former sporoblast gives rise to smaller and ordinary spores, while the latter gives rise to larger and abnormal spores.

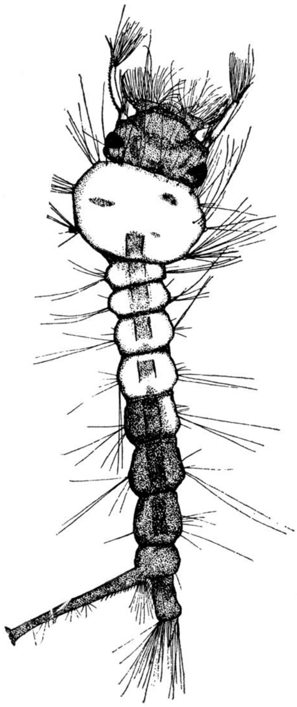

Figure A. — Dorsal view of larval *Culex apicalis* infected by *Thelohania opacita,* drawn soon after being killed with hot sublimate-alcohol-acetic acid mixture. The entire thorax and anterior four segments and a part of the fifth segment of the abdomen, show the characteristic appearance due to the presence of the parasites. × 11.

The spores are broadly ellipsoid and are circular in cross sections. One end is more rounded than the other. At the former extremity a clear space is usually present in mature spores. This space is rounded triangular, oval or circular in shape. The rest of the contents of the spore is uniformly and finely granulated. Dimensions: normal spores, 5.5 to 6μ long by 3.5 to 4μ broad, length of polar filament extruded by mechanical pressure 110μ; abnormal spores, 8 to 8.5μ long by 4.5 to 5.5μ broad (an exceptional one reaches a size of 10 by 5.7μ) ; length of polar filament 200μ.

The full morphology and development ot the species will be considered in later papers.

SUMMARY

1. Microsporidian infection often is lethal in its effect upon the mosquito larvae.
2. In captivity infected mosquito larvae die in much shorter time than uninfected ones.
3. Pupae and adults so far examined are free from the infection.
4. A new Microsporidian, *Thelohania opacita,* parasitic in *Culex apicalis* is described.
5. The geographical distribution of Microsporidia parasitic in mosquitoes is shown.

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